

Prevalence of hepatitis E virus in Italian pig herds. Preliminary results

Di Bartolo, I. ⁽¹⁾, Inglese, N. ⁽¹⁾, Pourshaban, M. ⁽¹⁾, Martelli, F. ⁽²⁾, Caprioli, A. ⁽³⁾, Ostanello, F. ⁽²⁾, Ruggeri, F. M. ^{*(1)}

⁽¹⁾ Dept. of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy;

⁽²⁾ Dept. of Veterinary Public Health and Animal Pathology, University of Bologna, Ozzano Emilia (BO), Italy;

⁽³⁾ Direzione Generale Sicurezza Alimentare e Nutrizione, Ministry for Health, Rome, Italy

* corresponding author: franco.ruggeri@iss.it

Abstract

Hepatitis E virus (HEV) is the causative agent of hepatitis E, and is an unenveloped positive sense single-stranded RNA virus. Swine HEV strains are genetically closely related to human strains from the same area, suggesting the occurrence of zoonotic transmission. Recently, human cases of hepatitis E have been linked to the consumption of raw or undercooked meat or organs from deer, wild boars or pigs. The disease is now considered an emerging food-borne transmitted zoonosis. During 2006, a pilot investigation was performed to determine the prevalence of HEV in pig farms located in Northern Italy. 274 faecal samples were collected from healthy fattening animals (3-4 and 8-9 months of age) and from healthy breeding animals (gilts and sows) from 6 different farms, and analyzed using a Nested-RT-PCR targeting the open reading frame 2 (ORF2) region. Stool samples were suspended in water, and viral RNA extraction was performed using a commercial kit. Extracted viral RNA was subjected to RT-PCR amplification using degenerate primers conA1-conS1 for the first amplification, and degenerate primers conA2-conS2 for the nested PCR, yielding a final fragment of 145 bp. HEV RNA was detected in sixty-nine of the 274 (25.2%) examined samples. None of the six farms resulted negative and the prevalence within the farms ranged between 2% and 60.5%. For the characterization of the strains, randomly selected positive samples were subjected to nucleotide sequencing, and aligned with those present in the NCBI Data Bank. Sequence analysis showed that all strains were Swine Hepatitis E belonging to Genotype 3. These preliminary results confirm that swine HEV is widespread in Italian swine farms.

Introduction

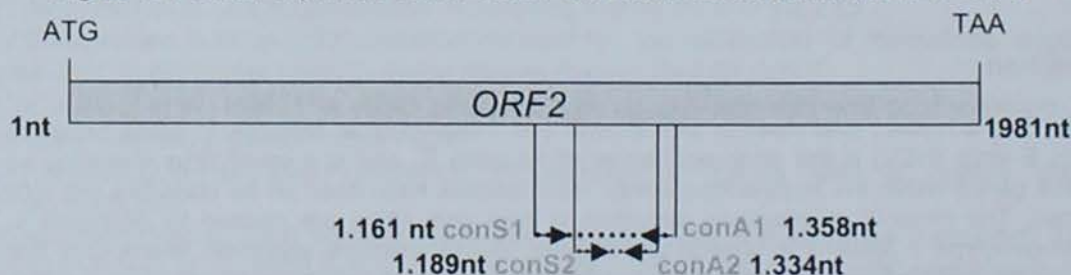
Hepatitis E is a human viral disease with clinical and morphological features of acute hepatitis. Hepatitis E virus (HEV) is the etiological agent of hepatitis E, and is a small virus classified as *Hepevirus* genus within the *Hepeviridae* family. HEV isolates have been so far classified into four genotypes. The majority of infections occurring in Asia and Africa are caused by genotype 1, whereas genotype 2 prevails in Mexico and Nigeria. In industrialized countries, where until few years ago the infection was considered non-endemic, only strains belonging to genotype 3 and 4 have been detected (Emerson and Purcell 2003). Genotype 3 prevails in USA and Europe, while genotype 4 is mainly distributed in Asia (Hsieh et al., 1999; Banks et al., 2004; Zheng et al., 2006). Although hepatitis E is a sporadic disease, in countries with good health-care systems the seroprevalence rate among healthy individuals can nonetheless be high (Emerson and Purcell 2003). The first animal HEV strain was characterized in pigs in USA in 1997 (Meng et al., 1997). Since then, several other swine strains have been described worldwide. In particular genotype 3 and 4 isolates from swine and humans from the same geographic area are often genetically closely related, suggesting that swine can represent a reservoir for the virus and that zoonotic transmission of HEV may play a relevant role in industrialised countries (Meng et al., 1998; Zanetti et al., 1999; Van der Poel et al., 2001; Clemente-Casares et al., 2003; Buti et al., 2004). Furthermore, several studies have reported that in people who work in contact with swine such as pig farmers, veterinarians and slaughterhouse workers, the HEV seroprevalence rate can be higher than in normal control populations (Meng et al., 2002; Hsieh et al., 1999). Recently hepatitis E cases have been linked to the consumption of raw or undercooked meat from deer, wild boars or

pigs, and the disease is now considered an emerging food-borne transmitted zoonosis (Matsuda et al., 2003; Tei et al., 2003; Yazaki et al., 2003). If the possibility of zoonotic transmission of the infection is accepted, it is clear that the higher the prevalence in animals, the greater the risk of transmission will be to humans. An evaluation of the prevalence and the genetic characterization of the HEV strains circulating in Italian pig farms will be therefore necessary to perform a risk assessment of zoonotic transmission in Italy. In this regard, this study was performed as a pilot investigation to evaluate the prevalence of HEV infection within farms in Northern Italy.

Material and Methods

Between January to August 2006, 274 faecal samples were collected from randomly selected pigs of 3-4 months of age (weaners), 8-9 months of age (fatteners), gilts (0 parities), young sows (1-2 parities) and old sows (> 2 parities) belonging to 6 different farms (2 farrow-to-weaning and 4 farrow-to-finish) located in Northern Italy, area of the country with the highest concentrations of pigs. To an external examination all the pigs sampled appeared clinically healthy. Faecal samples were collected directly from the rectum and placed in sterile containers. From each farm, the faeces of at least 10 animals for each production category were collected. This scheme provides a systematic sampling of different pig classes present within the herds and can estimate, with a 95% probability, the prevalence of swine HEV-positive animals with an expected prevalence of 30% and an accepted error of 25%. In order to detect the HEV genome, a nested-RT-PCR targeting a region of the ORF2 (coding for the HEV capsid protein) was performed. Total viral RNA was extracted from a faecal suspension (10%) using a QIAamp Viral kit (Qiagen). First round amplification utilized primers HEV ORF2 con-S1 (GACAGAATTRATTTTCGTCGGCTGG) and HEV ORF2 con-A1 (CTTGTTTCRTGYTGGTTRTCATAATC) yielding a product of 197 nucleotides. Nested reactions used primers HEV ORF2 con-S2 (GTGTCTCRGCCAATGGCGAGC) and HEV ORF2 con-A2 (GTTTCRTGYTGGTTRTCATAATCCTG) to produce a 145 nucleotide product (Erker et al., 1999) (figure 1). For further characterization, randomly selected positive samples coming from 3 different farms were subjected to nucleotide sequencing using ABI PRISM BigDye Terminator Kit 2.0 (Applied Biosystems). The sequenced fragments were aligned with those present in the NCBI Data Bank and analyzed using DNASIS Max software (Hitachisoft).

Figure 1. Position of primers conA1-conS1 and conA2-conS2 within the HEV ORF2



Results

HEV RNA was detected in 25.2% of the pigs sampled. None of the six farms resulted negative for HEV and the prevalence within the farms ranged between 2% and 60.5% (table 1). The 11 HEV strains considered for sequencing in this preliminary survey could be all placed within genotype 3 and shared 88.5 to 100% nucleotide sequence identity to each other. Sequence analysis showed that at least 6 different strains were circulating simultaneously in the farms considered. However, most of the nucleotide changes detected were found to represent silent mutations, and no differences at amino acid level were revealed.

Table 1. Prevalence of HEV infection in the 6 examined farms

Type of farm *	Farm n°	HEV positive/tested	(%)
F-F	1	1/50	2.0
F-W	2	6/40	15.0
F-F	3	12/48	25.0
F-F	4	6/47	12.8
F-F	5	21/51	41.6
F-W	6	23/38	60.5
All farms		69/274	25.2

* F-F= farrow-to-finish; F-W= farrow-to-weaning

Discussion

This investigation confirms that HEV is present in pigs in Italy (Caprioli et al., in press) and demonstrates that HEV is probably widely spread in Northern Italy swine farms. This study also demonstrates that not less than six distinct HEV Genotype 3 strains circulate in the country, suggesting that the repertoire of the swine HEV pool in Italy is probably as wide as described in other European countries (Van der Poel, 2001; Clemente-Casares et al., 2003; Banks et al., 2004; Buti et al., 2004). In this pilot investigation, none of the 6 examined farms resulted HEV free, and the mean prevalence observed was 25.6%, generally higher than the prevalence reported in other countries (Huang et al., 2002). Moreover, in our study, in contrast with previous works in which the infection was reported mainly to occur in animals of 3 to 5 months of age (Meng et al. 1997; Van der Poel, 2001; Banks et al., 2004), we found HEV positive animals distributed in all age classes. These findings, together with the observation that HEV infection is probably sub-clinical (all examined animals were apparently healthy) and was present also in pigs close to the slaughtering age, are of concern because of the risk of transmission of HEV to human beings by either contact with infected swine, infected carcasses or contaminated undercooked meat or organs (Yazaki et al., 2003). Moreover, the possibility of virus spread in the environment through manure from pig farms, with the consequent possible contamination of vegetables and drinking water, should also be taken into consideration. The finding of a widespread circulation of HEV in pig farms indicates the need of implementing surveillance systems and risk analysis programs to better clarify the role of swine as a HEV reservoir for human beings and the possible implications of this emerging zoonosis for public health in Italy.

References

- BANKS, M., HEATH, G.S., GRIERSON, S.S., KING, D.P., GRESHAM, A., GIRONES, R., WIDEN, F., HARRISON, T.J., 2004. Evidence For The Presence Of Hepatitis E virus in pigs in the United Kingdom. *Veterinary Record*, 154, 223-227.
- BUTI, M., CLEMENTE-CASARES, P., JARDI, R., FORMIGA-CRUZ, M., SCHAPER, M., VALDES, A., RODRIGUEZ-FRIAS, F., ESTEBAN, R., GIRONES, R., 2004. Sporadic cases of acute autochthonous hepatitis E in Spain. *Journal of Hepatology*, 41, 126-131.
- CAPRIOLI, A., MARTELLI, F., OSTANELLO, F., DI BARTOLO, I., RUGGERI, F., DEL CHIARO, L., TOLARI, F., 2007. Detection of Hepatitis E Virus (HEV) in Italian pig herd. *Veterinary Record* (in press).
- CLEMENTE-CASARES, P., PINA, S., BUTI, M., JARDI, R., MARTIN, M., BOFILL-MAS, S., GIRONES, R., 2003. Hepatitis E virus epidemiology in industrialized countries. *Emerging Infectious Disease*, 9, 448-454.
- EMERSON, S.U., PURCELL, R.H., 2003. Hepatitis E virus. *Reviews in Medical Virology*, 13, 145-154.
- ERKER, J.C., DESAI, S.M., SCHLAUDER, G.G., DAWSON, G.J., MUSHAHWAR, I.K., 1999. A hepatitis E virus variant from the United States: molecular characterization and transmission in cynomolgus macaques. *Journal of General Virology*, 80, 681-690.

- HSIEH, S.Y., MENG, X.J., WU, Y.H., LIU, S.T., TAM, A.W., LIN, D.Y., LIAW, Y.F., 1999. Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. *Journal of Clinical Microbiology*, 37, 3828-3834.
- HUANG, F. F., G. HAQSHENAS, D.K. GUENETTE, P.G. HALBUR, S.K. SHOMMER, F.W. PIERSON, T.E. TOTH, MENG, X.J., 2002. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. *Journal of Clinical Microbiology*, 40, 1326-1332.
- Matsuda, H., Okada, K., Takahashi, K., Mishiro, S. (2003). Severe hepatitis E virus infection after ingestion of uncooked liver from a wild boar. *Journal of Infectious Disease*, 188, 944.
- MENG, X.J., PURCELL, R.H., HALBUR, P.G., LEHMAN, J.R., WEBB, D.M., TSAREVA, T.S., HAYNES, J.S., THACKER, B.J., EMERSON, S.U., 1997. A novel virus in swine is closely related to the human hepatitis E virus. *Proc. National Academy of Sciences of the USA*, 94, 9860-9865.
- MENG, X.J., HALBUR, P.G., SHAPIRO, M.S., GOVINDARAJAN, S., BRUNA, J.D., MUSHAHWAR, I.K., PURCELL, R.H., EMERSON, S.U., 1998. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *Journal of Virology*, 72, 9714-9721.
- MENG, X.J., WISEMAN, B., ELVINGER, F., GUENETTE, D.K., TOTH, T.H., ENGLE, R.E., EMERSON, S.U., PURCELL, R.H., 2002. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *Journal of Clinical Microbiology*, 40, 117-122.
- TEI S., KITAJIMA N., TAKAHASHI K., MISHIRO S., 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet*, 362, 371-373.
- VAN DER POEL, W.H., VERSCHOOR, F., VAN DER HEIDE, R., HERRERA, M.I., VIVO, A., KOOREMAN, M., DE RODA HUSMAN, A.M., 2001. Hepatitis E virus sequences in swine related to sequences in humans, The Netherlands. *Emerging Infectious Disease*, 7, 970-976.
- YAZAKI, Y., MIZUO, H., TAKAHASHI, M., NISHIZAWA, T., SASAKI, N., GOTANDA, Y., OKAMOTO, H., 2003. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *Journal of General Virology*, 84, 2351-2357.
- ZANETTI, A. R., SCHLAUDER, G.G., ROMANÒ, L., TANZI, E., FABRIS, P., DAWSON, G.J., MUSHAHWAR, I.K., 1999. Identification of a novel variant of hepatitis E virus in Italy. *Journal of Medical Virology* 57, 356-360.
- ZHENG, Y., SHENGXIANG, G., ZHANG, J., GUO, Q., HON NG, M., WANG, F., XIA, N., JIANG, Q., 2006. Swine as a principal reservoir of Hepatitis E virus that infects Humans in Eastern China. *Journal of Infectious Disease*, 193, 1643-1649.